REMARKS

Applicants respectfully requests entry of the amendments and remarks submitted herein. Claims 30, 39 and 48 are amended, and claim 59-72 are added. Therefore, claims 30, 34, 37-39, 43, 46-48 and 54-72 are currently pending.

Support for the amendments to claims 30, 39 and 48 are found throughout the specification. For example, support for the *rfe* gene being endogenous to *Salmonella* bacterial production cell can be found on page 4, line 29 through page 5, line, 3 (which implies that the production cell can endogenously contain an *rfe* gene).

Support for the amendment to claims 30, 39 and 48 reciting naturally-occurring LsgG can be found throughout the specification, such as, for example, at page 10, lines 4-20 (Hib strain was isolated from an individual with meningitis).

Support for new claims 59-72 reciting LsgG encoded by pGEMLOS-4, pGEMLOS-5 or pGEMLOS-7 can be found throughout the specification, such as, for example, in Examples 3, 4, and 6 and the Tables.

Rejection under 35 U.S.C. §112, First Paragraph

The examiner rejected claims 30, 34, 37-39, 43, 46-48 and 54-58 under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. The claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention.

Independent claim 30 recites a process for the production of a lipooligosaccharide (LOS) which comprises the steps of (a) growing in a culture medium Salmonella minnesota bacteria comprising (i) a core lipid structure containing a terminal heptose and (ii) a DNA sequence encoding an Undecaprenyl-phosphate N-acetyl glucosaminyl phosphate transferase (rfe) that is part of the Salmonella minnesota genome, and (iii) an isolated DNA sequence encoding a naturally-occurring lipooligosaccharide-synthesis gene G polypeptide (LsgG) from Haemophilus influenzae, wherein the DNA sequence encoding rfe is regulated by LsgG such that the LOS is synthesized by the addition of an acceptor molecule to the terminal heptose molecule; and (b)

recovering the LOS from the culture medium. Claims 34, 37-38 and 56 depend either directly or indirectly from claim 30.

Independent claim 39 recites a process for the production of a complex carbohydrate comprising the steps of (a) growing in a culture medium Salmonella minnesota bacteria comprising (i) a core lipid structure containing a terminal heptose and (ii) a DNA sequence encoding an Undecaprenyl-phosphate N-acetyl glucosaminyl phosphate transferase (rfe) that is part of the Salmonella minnesota genome, and (iii) an isolated DNA sequence encoding a naturally-occurring lipooligosaccharide-synthesis gene G polypeptide (LsgG) from Haemophilus influenzae, wherein the DNA sequence encoding rfe is regulated by LsgG such that a complex carbohydrate is synthesized by the addition of an acceptor molecule to the heptose molecule; and (b) recovering the complex carbohydrate from the culture medium. Claims 43, 46-47 and 57 depend either directly or indirectly from claim 39.

Independent claim 48 recites a method comprising modifying a terminal heptose of a lipopolysaccharide (LPS) or lipooligosaccharide (LOS) core structure of a Salmonella minnesota bacterium, wherein the bacterium comprises a polynucleotide encoding an Undecaprenyl-phosphate N-acetyl glucosaminyl phosphate transferase (rfe) that is part of the Salmonella minnesota genome and an isolated naturally-occurring DNA sequence encoding a lipooligosaccharide-synthesis gene G polypeptide (LsgG) from Haemophilus influenzae, wherein the polynucleotide encoding rfe is regulated by lipooligosaccharide-synthesis gene G polypeptide (LsgG) from Haemophilus influenzae such that an N-acetyl glucosamine is added onto the terminal heptose so as to modify the terminal heptose. Claims 54-55 and 58 depend either directly or indirectly from claim 48.

Independent claim 59 recites a process for the production of a lipooligosaccharide (LOS) which comprises the steps of: (a) growing in a culture medium *Salmonella minnesota* bacteria comprising (i) a core lipid structure containing a terminal heptose and (ii) a DNA sequence encoding an Undecaprenyl-phosphate N-acetyl glucosaminyl phosphate transferase (*rfe*) that is part of the *Salmonella minnesota* genome, and (iii) an isolated DNA sequence encoding a lipooligosaccharide-synthesis gene G polypeptide (LsgG) from *Haemophilus influenzae*, wherein the DNA sequence encoding *rfe* is regulated by LsgG such that the LOS is synthesized by the addition of an acceptor molecule to the terminal heptose molecule, and wherein the isolated DNA sequence encoding a lipooligosaccharide-synthesis gene G polypeptide (LsgG) from

Haemophilus influenzae is encoded by pGEMLOS-4, pGEMLOS-5 or pGEMLOS-7; and (b) recovering the LOS from the culture medium. Claims 60-63 depend either directly or indirectly from claim 59.

Independent claim 64 recites a process for the production of a complex carbohydrate comprising the steps of: (a) growing in a culture medium *Salmonella minnesota* bacteria comprising (i) a core lipid structure containing a terminal heptose and (ii) a DNA sequence encoding an Undecaprenyl-phosphate N-acetyl glucosaminyl phosphate transferase (*rfe*) that is part of the *Salmonella minnesota* genome, and (iii) an isolated DNA sequence encoding a lipooligosaccharide-synthesis gene G polypeptide (LsgG) from *Haemophilus influenzae*, wherein the DNA sequence encoding *rfe* is regulated by LsgG such that a complex carbohydrate is synthesized by the addition of an acceptor molecule to the heptose molecule, and wherein the isolated DNA sequence encoding a lipooligosaccharide-synthesis gene G polypeptide (LsgG) from *Haemophilus influenzae* is encoded by pGEMLOS-4, pGEMLOS-5 or pGEMLOS-7; and (b) recovering the complex carbohydrate from the culture medium. Claims 65-68 depend either directly or indirectly from claim 64.

Independent claim 69 recites a method comprising modifying a terminal heptose of a lipopolysaccharide (LPS) or lipooligosaccharide (LOS) core structure of a *Salmonella minnesota* bacterium, wherein the bacterium comprises a polynucleotide encoding an Undecaprenyl-phosphate N-acetyl glucosaminyl phosphate transferase (*rfe*) that is part of the *Salmonella minnesota* genome and an isolated DNA sequence encoding a lipooligosaccharide-synthesis gene G polypeptide (LsgG) from *Haemophilus influenzae*, wherein the polynucleotide encoding *rfe* is regulated by lipooligosaccharide-synthesis gene G polypeptide (LsgG) from *Haemophilus influenzae* such that an N-acetyl glucosamine is added onto the terminal heptose so as to modify the terminal heptose, and wherein the isolated DNA sequence encoding a lipooligosaccharide-synthesis gene G polypeptide (LsgG) from *Haemophilus influenzae* is encoded by pGEMLOS-4, pGEMLOS-5 or pGEMLOS-7. Claims 70-72 depend either directly or indirectly from claim 69.

The examiner indicates that the specification does not provide adequate support for the transformation of bacteria with any or all polynucleotides encoding an *rfe* including any or all variants, mutants and recombinants thereof. Applicant disagrees with this statement. In order to further prosecution, however, applicant has amended the claims to recite that the DNA sequence encoding an Undecaprenyl-phosphate N-acetyl glucosaminyl phosphate transferase (*rfe*) is part

of the *Salmonella minnesota* genome. The claims have also been amended to recite that the LsgG is encoded by an isolated DNA sequence encoding a naturally-occurring lipooligosaccharide-synthesis gene G polypeptide (LsgG) from *Haemophilus influenzae*, or that the *LsgG* lipooligosaccharide-synthesis gene G polypeptide (LsgG) is encoded by an isolated DNA sequence encoding a naturally-occurring pGEMLOS-4, pGEMLOS-5 or pGEMLOS-7.

Applicant, therefore, requests that this rejection under 35 U.S.C. §112, first paragraph (written description) be withdrawn.

Rejection under 35 U.S.C. §103(a)

The examiner rejected claims 30, 34, 37-39, 43, 46-48 and 54-58 under 35 U.S.C. §103(a) as being unpatentable over McLaughlin et al. (*Journal of Endotoxin Research*, *1*, 165-174 (1994); hereinafter McLaughlin) in view of Preston et al. (*Critical Reviews in Microbiology*, *22*, 139-180 (1996); hereinafter Preston) and Swierzko et al. (*Infection and Immunity*, *61*, 3216-3221 (1993); hereinafter Swierzko). The examiner also cited Brade et al. (*Infection and Immunity*, 55:482-486 (1987); hereinafter Brade) and Alexander et al. (*J. Bacteriol.*, 176:7079-7084 (1994); hereinafter Alexander) in the rejection.

A rejection of obviousness under 35 U.S.C. § 103 requires that the Examiner establish a *prima facie* case of obviousness. To establish a *prima facie* case of obviousness, the Examiner has the burden to establish three basic elements. First, the Examiner must establish that there is some suggestion or motivation, either in the cited documents themselves or in the knowledge generally available to an art worker, to modify the documents or to combine document teachings so as to arrive at the claimed invention. Second, the Examiner must establish that there is a reasonable expectation of success. Finally, the Examiner must establish that the prior art documents teach or suggests all the claim limitations. M.P.E.P. 2143.

McLaughlin discusses the sequence of the *lsg* locus from *H. influenzae*. They identified eight open reading frames (ORFs) and performed transposon mutagenesis to try to begin to determine what, if any, function the products deduced from the ORF sequences might have. The authors of the paper did not have success in identifying the function of the deduced protein sequences encoded by the ORFs.

The Examiner asserts that McLaughlin teach that various sugar transferases expressed from the lsg are responsible for the modification of the existing *E. coli* LPS (page 172).

Applicants would like to draw the Examiner's attention to the full passage at page 172 of McLaughlin, which states the following:

> [I]t is most probably various sugar transferases expressed from the Hib *lsg* locus that are responsible for these modification[s] of the existing *E. coli* LPS. . . . Thus, it is likely that the *lsg* locus should contain a series of genes coding for sugar transferases. The sequence analysis of the 7.4 kb fragment and database search for the proteins homologous with the 8 ORFs, however, failed to show significantly high homology to any known sugar transferase, and it was not possible to deduce the functions of the products of the ORFs based on the sequence homology." (Emphasis added)

McLaughlin later states that "future studies will be directed at defining the functions of the proteins expressed by the ORFs within this locus" (page 174). Thus, when the statement quoted by the examiner is read in context of the full passage from McLaughlin, it becomes clear that McLaughlin expected one or more of the ORFs to encode a sugar transferase, but that they could not find any significantly high homology to any known sugar transferase. Thus, at the time of publication, they could not determine the functions of the putative products encoded by the ORFs.

Contrary to the statement on page 8 of the July 2, 2007 Office Action, there is no teaching in McLaughlin that "In the method of McLaughlin et al., N-acetyl glucosamine is added to terminal heptose of a LOS or LPS core structure." Since there is no teaching in McLaughlin regarding the putative products encoded by the ORFs, one would not have been motivated to isolate one of the ORFs, and transform it into a different type of bacteria. Applicants respectfully assert that it was not until the experiments were performed by the present inventors that it was discovered that the LsgG protein was encoded by one of the eight ORFs. As discussed above, McLaughlin could not determine the functions of the putative products encoded by the ORFs. Thus, McLaughlin could not teach which specific ORF encoded LsgG. This information would be needed before the ORF could be isolated and then used to product the LOS (claim 30) or complex carbohydrate (claim 39), or to modify a terminal heptose of a Lipopolysaccharide (LPS) or Lipooligosaccharide (LOS) core structure (claim 48).

Further, it would not have been obvious to substitute *Salmonella* for *E. coli*. It was quite surprising that one could start with a *Salmonella* bacterium, transform in a gene from another type of bacteria (*i.e.*, the *LsgG* gene), and have the foreign protein, LsgG, successfully regulate

another gene (*rfe*) such that an acceptor molecule is added to a core region to make the chimeric carbohydrate. In nature, *Salmonella* does not place any additional sugars onto the core region. Therefore, it was unexpected that by simply transforming a regulatory gene into *Salmonella*, the bacterium would produce the chimeric carbohydrate. Nothing in McLaughlin suggests that it would have been desirable or advantageous, or even suitable, to have expressed the *H. influenzae* lipooligosaccharide synthesis gene *LsgG* in *Salmonella*, as presently claimed. Absent some motivation for modifying the prior art, the mere fact that the prior art could have been modified in the claimed manner does not render the claimed subject matter obvious.

Swierzko does not remedy the deficiencies of McLaughlin. Swierzko discloses the serological characterization of antisera collected from rabbits immunized with heat-killed *Salmonella minnesota* R4 Chemotype Rd_2P^- (see Abstract and page 3218). They extracted LPS from *Salmonella minnesota* R4 chemotype Rd_2P^- and made synthetic antigens (page 3216-17). Swierzko investigated "the immunogenic and antigenic properties of RD chemotype LPS carrying heptosyl residues in terminal positions" (p. 3216). Swierzko, in a passive hemolysis assay, disclose that the smallest structure recognized by the antisera was Hep-Kdo-GlcNhm2, and that the smallest structure reacting in an EIA was Hep-Kdo disaccharide. However, there is nothing in Swierzko that teaches or suggests a "core lipid structure containing a terminal heptose."

Preston does not remedy the deficiencies of McLaughlin and Swierzko. The Examiner states that Preston "discloses several genes involved in LOS biosynthesis, including the lsg gene and rfe gene isolated from *H. influenzae* (Table page 154)," and that Preston teaches "that *H. influenzae* produce LOS lacking O-antigens, even though O-antigens are present in LPS produced by most Gram-negative bacteria." Applicant respectfully reminds the Examiner, however, that *lsg* is a locus, and not just an individual gene. Preston does not specify which gene, or group of genes, in the *lsg* locus is involved in the addition of an acceptor molecule onto the terminal heptose of a core lipid structure. Preston does not teach or suggest any further information about the various genes in this locus, and certainly not a specific isolated DNA sequence encoding a lipooligosaccharide-synthesis gene G polypeptide (LsgG), as recited in the pending claims. Further, Preston does not tech or suggest selecting the *lsg* locus out the many other identified LOS biosynthesis genes listed in Table 4.

Brade does not remedy the deficiencies of McLaughlin, Swierzko and Preston. The examiner cites to Brade for the proposition that one of ordinary skill in the art could transform *Salmonella*. Brade, however, does not teach the function of LsgG in regulating *rfe* such that an acceptor molecule is added to the terminal heptose molecule of a core lipid structure in *Salmonella*.

Alexander does not remedy the deficiencies of McLaughlin, Swierzko, Preston and Brade. Alexander relates to the role *rfe* plays in the formation of O-specific polysaccharide and ECA. There is nothing in either McLaughlin or Alexander that teaches or suggests that LsgG regulates *rfe* such that an acceptor molecule is added to the terminal heptose molecule of a core lipid structure.

Applicants respectfully submit that the Examiner has not established a motivation, nor the expectation of success, for using *Salmonella minnesota* bacteria that contain: (1) a core lipid structure containing a terminal heptose; (2) a DNA sequence encoding an Undecaprenyl-phosphate N-acetyl glucosaminyl phosphate transferase (*rfe*); and (3) an isolated sequence encoding a lipooligosaccharide-synthesis gene G polypeptide (LsgG) from Haemophilus influenzae, wherein the DNA sequence encoding rfe is regulated by LsgG such that the LOS is synthesized by the addition of an acceptor molecule to the terminal heptose molecule for producing LOS, and recovering the LOS from the culture medium (claim 30) or complex carbohydrate (claim 39) or a method comprising modifying a terminal heptose of a LPS or LOS core structure of a gram-negative bacterial species, wherein the gram-negative bacterial species comprises a polynucleotide encoding *rfe* and an isolated DNA sequence encoding a LsgG from *Haemophilus influenzae*, wherein the polynucleotide encoding *rfe* is regulated by LsgG such that an N-acetyl glucosamine is added onto the terminal heptose so as to modify the terminal heptose, wherein the gram-negative bacterial species is *Salmonella minnesota* (claim 48). In particular, the Examiner has not provided the motivation to specifically transform *Salmonella minnesota* bacteria with *Haemophilus influenzae* LsgG so as to perform the recited methods.

Applicants respectfully submit that the Examiner has not demonstrated that the claims are *prima facie* obvious in view of the cited documents, for example, because the Examiner has not established the suggestion or motivation, either in the cited documents themselves or in the knowledge generally available to an art worker, to modify the documents or to combine document teachings so as to arrive at the claimed invention. Therefore, Applicants respectfully

request the withdrawal if this rejection of claims 30, 34, 37-39, 43, 46-48 and 54-58 under 35 U.S.C. §103(a).

CONCLUSION

The Examiner is invited to contact Applicant's Representative at the below-listed telephone number if there are any questions regarding this Response or if prosecution of this application may be assisted thereby. If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 50-3503. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extension fees to Deposit Account 50-3503.

Respectfully submitted,

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